1. **Objective**
   This protocol describes the procedure for immunostaining of tissues grown in the OrganoPlate® 2-lane and 3-lane (see chip layout below).

2. **Background**
   Immunofluorescent staining is a technique that uses antibodies to target specific cellular biomolecules expressed on cells. Detection of the bound antibodies is done using fluorescence microscopy.

3. **Materials**
   - Tween-20 (Sigma, cat# P9616)
   - FBS (Gibco/ATCC, cat# A13450)
   - Triton™ X-100 (Sigma, cat# T8787)
   - HBSS (+Ca/Mg) (Sigma, cat# 55037C-1000ML)
   - BSA (Sigma, cat# A2153)
   - Crushed ice
   - Repeating multichannel pipets and tips
   - 1x PBS (Gibco, cat# 70013065)
   - Rocker platform (optional)
   - Fixative (see table 1)
     - Standard fixative is 3.7% formaldehyde (diluted 1:10 from stock, Sigma, cat# 252549-1L)

<table>
<thead>
<tr>
<th>Fixative</th>
<th>Incubation time</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.7% formaldehyde in HBSS</td>
<td>10-15min RT</td>
</tr>
<tr>
<td>0.4% formaldehyde in HBSS</td>
<td>10-15min RT</td>
</tr>
<tr>
<td>-20°C 100% acetone</td>
<td>5min RT</td>
</tr>
<tr>
<td>-20°C 100% methanol</td>
<td>10-15min RT</td>
</tr>
<tr>
<td>-20°C 95% methanol, 5% acetic acid</td>
<td>5-10min RT</td>
</tr>
</tbody>
</table>

   **Table 1.** Fixation methods compatible with the OrganoPlate®

   - Permeabilization buffer: 0.3% Triton X-100
   - Blocking solution: 2% FBS, 2% BSA, 0.1% Tween20 in PBS
   - Washing solution: 4% FBS in PBS
   - Antibodies and nuclear stain (Hoechst™/DraQ5™)
4. Assay

Fixation

All steps are performed at room temperature

1. Prepare fixative (see table 1)
   a. Standard fixative is 3.7% formaldehyde in HBSS (dilute 1:10 from 37% stock)
   b. Complete OrganoPlate® 2-lane: 21 mL
   c. Complete OrganoPlate® 3-lane: 17 mL

2. Aspirate medium from the chips and add fixative to the chips’ inlets and outlets according to the volume scheme below
   a. OrganoPlate® 2-lane: 50 µL to gel inlet, 100 µL to medium inlet, 50 µL to medium outlet
   b. OrganoPlate® 3-lane: 100 µL to top medium inlet, 50 µL to all other inlets and outlets

3. Incubate the fixative for the appropriate amount of time (see table 1)

4. Aspirate fixative and wash chips 2x (5 min each) with PBS using the volume scheme shown in step 2

5. Proceed to immunostaining or seal the plate around the edges with Parafilm® and wrap the plate in aluminum foil. The plate can be stored at RT up to 2 weeks.

Immunostaining

All steps are performed at room temperature unless specified otherwise

To allow successful binding of primary and secondary antibodies, antibody is perfused through the OrganoPlate® during antibody incubation steps. Perfusion can be created by placing the OrganoPlate® on a regular rocker platform and having it switch sides. Use a small angle and a low switching interval (i.e. 5° angle, 2-5 min interval). Alternatively, flow can be induced by placing the OrganoPlate® under an angle by positioning one end on top of an object (see figure on the right) and regularly switching sides.

6. Prepare permeabilization buffer, blocking solution, and washing solution (see materials list)

7. Wash chips 1x 5 min with washing solution according to the volume scheme below
   a. OrganoPlate® 2-lane: 50 µL to gel inlet, 100 µL to medium inlet, 50 µL to medium outlet
   b. OrganoPlate® 3-lane: 100 µL to top medium inlet, 50 µL to all other inlets and outlets

8. Permeabilize cells for 10 minutes with permeabilization buffer according to the volume scheme shown in step 7
9. Wash chips 1x 5 min with **washing solution** using the volume scheme shown in step 7
10. Block cells for 30-45 min with **blocking solution** according to the volume scheme shown in step 7
11. Meanwhile, prepare primary antibody in blocking solution in the appropriate dilutions
    a. OrganoPlate® 2-lane: 40 µL antibody per chip
    b. OrganoPlate® 3-lane: 80 µL antibody per chip
12. Aspirate blocking solution and add **primary antibody solution** according to the volume scheme below

```
<table>
<thead>
<tr>
<th></th>
<th>25µL</th>
<th>25µL</th>
</tr>
</thead>
<tbody>
<tr>
<td>0µL</td>
<td>0µL</td>
<td>0µL</td>
</tr>
<tr>
<td>15µL</td>
<td>15µL</td>
<td>15µL</td>
</tr>
</tbody>
</table>
```

13. Incubate primary antibody on the rocker platform at RT (or at 4°C if desired)
    a. For tubular cultures, 1-2 hours of incubation on the rocker platform at RT is sufficient
    b. For cultures of cells in gel, longer incubation times may be required (see page 4)
14. Meanwhile, prepare secondary antibody in blocking solution in the appropriate dilutions
    a. OrganoPlate® 2-lane: 40 µL antibody per chip
    b. OrganoPlate® 3-lane: 80 µL antibody per chip
15. Wash chips 2x (3 min each) with **washing solution** using the volume scheme shown in step 7
16. Add **secondary antibody solution** according to the volume scheme shown in step 12
17. Incubate secondary antibody in the dark on the rocker platform at RT
    a. For tubular cultures, 30 minutes of incubation on the rocker platform at RT is sufficient
    b. For cultures of cells in gel, longer incubation times may be required (see page 4)
18. Wash chips 2x (3 min each) with **washing solution** using the volume scheme described in step 7
19. If desired, stain cells with direct stains (e.g. Hoechst or ActinRed), using manufacturer’s instructions
    a. Use stains for fixed cells
    b. Use the volume scheme shown in step 12
    c. Incubate stains on the rocker at RT at least 15 min for cells grown as tubes against the ECM gel and at least 30 min for cells embedded in ECM gel (see page 4)
20. Wash chips 1x (5 min) with **PBS** according to the volume scheme shown in step 7
21. Aspirate all wells and add 50 µL of **PBS** to all wells

```
<table>
<thead>
<tr>
<th>50µL</th>
<th>50µL</th>
<th>50µL</th>
</tr>
</thead>
<tbody>
<tr>
<td>50µL</td>
<td>50µL</td>
<td>50µL</td>
</tr>
</tbody>
</table>
```

22. Proceed to microscopy or store the plate
    a. Perform microscopy within one week after staining for optimal results
    b. Imaging can be performed on all standard fluorescent microscopes
    c. Store plate by sealing edges with Parafilm® and wrapping it in aluminum foil. Store at RT for up to two weeks
5. Troubleshooting

Insufficient staining

Depending on the setup of the culture, some cultures may require longer staining procedures to obtain optimal results, for example neuronal networks grown in Matrigel® in the OrganoPlate® 2-lane. For these types of cultures, the following measures can be taken to improve antibody staining:

- Addition of 1% Triton to the primary and secondary antibody solution (steps 11 & 14)
- Prolongation of incubation times
  - Incubate primary antibody for 2 days, on the rocker, at RT (step 13b)
  - Incubate secondary antibody for 2 days, on the rocker, at RT (step 17b)
  - Incubate nuclear stains for 1 day, on the rocker, at RT (step 19c)

*Note: because these measures prolong the staining procedure significantly, use of aseptic technique is recommended to avoid growth of bacteria in the cultures. After fixation, work in a sterile cabinet and use sterile solutions (prepare solutions using only sterile components or filter buffers after preparation).*
# MIMETAS product list

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Product Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>MI-AR-CC-01</td>
<td>OrganoReady® Caco-2</td>
</tr>
<tr>
<td>9605-400-B</td>
<td>OrganoPlate® 2-lane</td>
</tr>
<tr>
<td>4004-400-B</td>
<td>OrganoPlate® 3-lane 40</td>
</tr>
<tr>
<td>6405-400-B</td>
<td>OrganoPlate® 3-lane 64</td>
</tr>
<tr>
<td>6401-400-B</td>
<td>OrganoPlate® Graft</td>
</tr>
<tr>
<td>MI-OFPR-S</td>
<td>OrganoFlow® S</td>
</tr>
<tr>
<td>MI-OFPR-L</td>
<td>OrganoFlow® L</td>
</tr>
<tr>
<td>MI-OT-1</td>
<td>OrganoTEER®</td>
</tr>
</tbody>
</table>

# Contact information

For questions, please contact us through the e-mail addresses stated below

**Purchasing:** order@mimetas.com

**Customer service:** info@mimetas.com

**Technical support:** support@mimetas.com

---

**MIMETAS Europe**  
J.H. Oortweg 19  
2333 CH, Leiden  
The Netherlands  
Phone: +31 (0)85 888 3161

**MIMETAS USA**  
704 Quince Orchard Road  
Suite 260, MD 20878  
Gaithersburg, USA  
+1 (833) 646-3827

**MIMETAS Japan**  
4F Tekko Building,  
1-8-2 Marunouchi, Chiyoda-Ku  
Tokyo, 100-0005, Japan  
+81 3-6870-7235

---

This protocol is provided 'as is' and without any warranties, express or implied, including any warranty of merchantability or fitness for a particular purpose or assured results, or that the use of the protocol will not infringe any patent, copyright, trademark, or other proprietary rights. This protocol cannot be used for diagnostic purposes or be resold. The use of this protocol is subject to Mimetas’ General Terms and Conditions of Delivery, Purchase and Use.

MIMETAS®, OrganoPlate®, OrganoFlow®, OrganoReady®, and OrganoTEER® are registered trademarks of MIMETAS BV. Parafilm® is a registered trademark of Bemis Company, Inc. Matrigel® is a registered trademark of Corning, Inc.